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ORIGINAL ARTICLE

Marker-assisted introgression of the major bacterial blight resistance gene, *Xa21* and blast resistance gene, *Pi54* into RPHR-1005, the restorer line of the popular rice hybrid, DRRH3

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Abstract The elite Indian rice hybrid, DRRH3 is highly susceptible to two major diseases, bacterial blight (BB) and blast, which limit its productivity significantly. In the present study, we have introgressed two major genes, viz., *Xa21* and *Pi54* conferring resistance against BB and blast, respectively into RPHR-1005, the male parent of DRRH3 through marker-assisted backcross breeding (MABB) and analyzed the backcross derived plants for their resistance against BB and blast. RPBio Patho-2 was used as a donor for both the resistance genes. Gene-specific markers were used for the foreground selection of *Xa21* and *Pi54* at each stage of backcrossing and markers specific for the major fertility restorer genes, *Rf3* and *Rf4* were used only at BC_1F_1 generation for foreground selection. Background selection was done using 62 polymorphic SSR markers and marker-assisted backcrossing was continued till BC_3 generation. At BC_3F_4, through intensive phenotype-based selections 15 promising lines (ABLs) possessing high level of resistance against BB and blast, high yield, fine-grain type, complete fertility restoration along with better panicle exsertion and taller plant type as compared to RPHR-1005 were identified and test crossed with APMS 6 A, the female parent of DRRH3. The newly derived hybrids (i.e. improved versions of DRRH3) were observed to possess high level of resistance against BB and blast along with medium-slender grain type and yield level better than or equivalent to that of DRRH3. Our study exemplifies the utility of MABB for targeted improvement of multiple traits in hybrid rice.

Keywords Bacterial blight resistance · Blast resistance · Marker-assisted backcross breeding · RPHR-1005 · DRRH3 · Fertility restoration · *Rf4* · *Rf3*

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Introduction

Rice (*Oryza sativa L.*) is the principal food crop of the world and India and currently feeds more than one third of the world's population and more than half of India's population. In order to keep pace with the growing population in India, the estimated rice requirement by 2025 is about 130 mt (Spielman et al. 2013). Among the various technological options available for increasing rice production, large-scale adoption of hybrid rice is one of the most promising approaches (Ahmed and Siddiq 1998). Hybrid rice plays a pivotal role in increasing rice production worldwide as they have a yield advantage

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of about 15–20 % over the high-yielding inbred varieties (Yuan 1994, Normile 1999). More than 70 public and private-bred hybrids have been released in India occupying ~2.5 mha (ICAR, 2015, Progress Report).

Among the hybrids released so far in India, DRRH3 developed and released by Indian Institute of Rice Research (IIRR), Hyderabad, India is very unique as it is the first hybrid possessing the highly-preferred, medium-slender grain type. Despite, its popularity, DRRH3 and its parental lines, APMS 6 A (female parent) and RPHR-1005 (male parent) are highly susceptible to two of the most devastating diseases, bacterial blight (BB) and blast, which limit the spread of the hybrid considerably (Viraktamath et al. 2010). It (despite its high susceptibility to many pests and diseases) has been recommended for cultivation in States such as Andhra Pradesh, Madhya Pradesh, Orissa, Uttar Pradesh and Gujarat by Government of India as a National Release. The grain quality traits of the hybrid are similar to that of a popular variety Samba Mahsuri (BPT-5204) with higher milling (>71 %), head rice recovery (>60 %), L/B ratio (2.61), intermediate amylose content (24 %) and gel consistency of 63 mm, strong culm and has superior performance even under lower doses of N (40 kg N/ha) indicating its higher nitrogen use efficiency and produce about 23–30 % more yield than BPT 5204 with comparable quality features. Hence, it will be desirable to incorporate at least one or more genes conferring resistance against the two diseases in the restorer parent so that not only DRRH3, but also any other hybrid developed using improved versions of RPHR-1005 will be disease resistant.

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the serious threats to the rice crop in irrigated and rainfed areas of the world (Mew, 1987). Numerous studies have been carried out related to the disease diagnosis, management and control. Enhancement of genetic resistance has proven to be the most effective method for controlling the disease (Khan et al. 2014). Till date, at least 40 BB resistance (both dominant and recessive) genes have been identified (Bhasin et al. 2012; Natrajkumar et al. 2012) and designated in a series from *Xa1* to *Xa40* (Yang et al. 1998; Sun et al. 2003; Gu et al. 2005; Cheema et al. 2008; Kim et al. 2015). Of these, *Xa21*, a major resistance gene, originally introgressed from *Oryza longistaminata* was observed to confer resistance to most Indian isolates of the bacterial pathogen and a highly efficient PCR-based marker called pTA248, developed by Ronald et al. (1992) is available for marker-assisted selection of the gene.

Rice blast, caused by the fungus *Magnaporthe oryzae*, leads to significant rice yield loss in all major rice-growing regions of the world (Khush and Jena 2009). Host plant resistance is the most effective strategy for management of blast disease and till now, at least 100 rice blast resistance genes (R-genes) have been identified (Sharma et al. 2012). Among these, *Pi54*, a major blast resistant gene from the Vietnamese cultivar, Tetep has been identified to be highly effective under Indian conditions (Sharma et al. 2010). Tightly linked and gene-specific markers are available for *Pi54* (Sharma et al. 2005; Ramkumar et al. 2011).

Based on the above mentioned points, the present study was conceptualized and carried out with an objective to introgress major, dominant resistance genes each conferring resistance for BB (i.e. *Xa21*) and blast (i.e. *Pi54*) into the genetic background of RPHR-1005 through MABB strategy and development of disease resistant versions of the restorer RPHR-1005 and its hybrid, DRRH3.

Materials & Methods

Plant material

An introgression line in the genetic background of Samba Mahsuri, RPBio Patho-2 (Prasad et al. 2011), possessing medium slender grain type, derived from the cross, Improved Samba Mahsuri (ISM) and Tetep, possessing, BB resistance gene, *Xa21* (from ISM) and Blast resistance gene, *Pi54* (from Tetep) was used as the donor. RPHR-1005, a stable restorer line, possessing medium-slender grain type, derived from the cross BPT5204/SC_5 126–3-2-4 (Ramesha et al. 2010) was used as the recipient parent. In addition, Taichung Native 1(TN1), HR12 were used as susceptible checks, for BB and blast resistance respectively.

Marker-assisted breeding strategy

RPHR-1005 was initially crossed with RPBio Patho-2 and 'true' F_1 plants were identified with the help of *Xa21* specific co-dominant marker pTA248 (Ronald et al. 1992) and *Pi54* (Ramkumar et al. 2011) gene-specific marker Pi54MAS were then backcrossed with RPHR-1005 to generate BC_1F_1s, which were then confirmed for the presence of resistance allele(s) of *Xa21* and *Pi54* in heterozygous condition and presence of fertility linked alleles with respect to the major fertility restorer genes, *Rf3* and *Rf4* using the gene-specific markers DRRM-RF3-10 and DRCG-RF4-14, respectively (Balaji Suresh et al., 2012). MABB strategy (Supplementary Fig. 1) was continued till BC_3 generation, wherein a single BC_3F_1 plant 'positive' for the target genes and possessing maximum recovery of the recurrent parent genome was selfed to generate BC_3F_2s,. The homozygous positive lines were further selected based on agro-morphological parameters

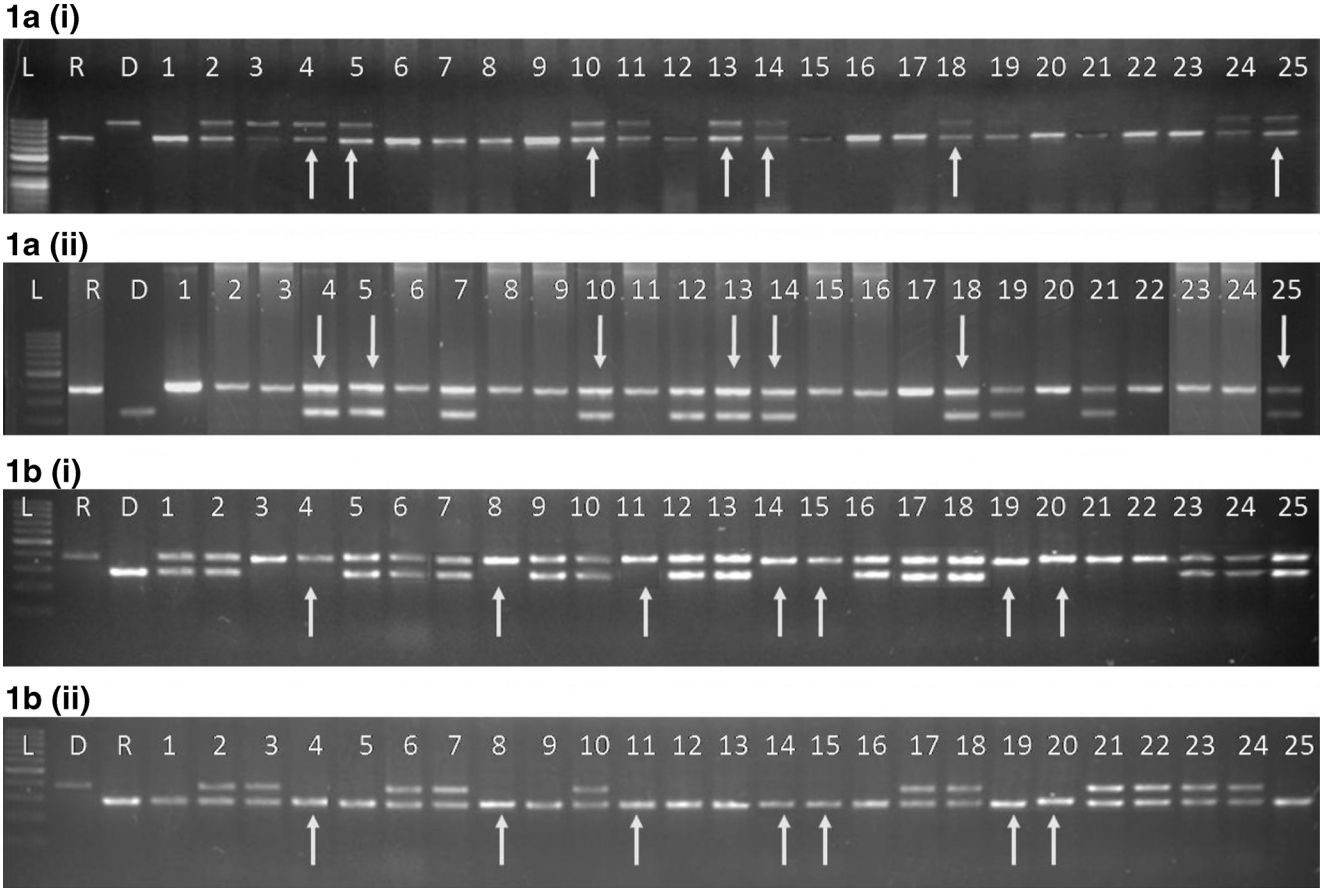

Fig. 1 Marker-assisted foreground selection at BC_1F_1 generation for *Xa21* [1 a (i).], *Pi54 [1a (ii)]*, *Rf3* [1b (i)] and *Rf4* [1b (ii)]. While heterozygous plants were selected for *Xa21* and *Pi54* based on gene-specific markers, plants homozygous for the restorer allele (determined based on marker analysis) were selected with respect to *Rf3* and *Rf4*. as Lanes L: 100 bp molecular weight ladder; D- Donor parent (RPBio Patho-2), R- Recurrent parent (RPHR-1005); 1–25 – BC_1F_1 plants; Arrows indicated 'positive plants'. Plant # 4 and 14 were positive for all the four genes

till BC_3F_5 generation and the selected ABLs were subjected for screening against BB (BC_3F_4) and blast (BC_3F_5) diseases.

Polymerase chain reaction for foreground and background selection

Mini scale DNA isolation of parents and backcross derived lines was carried out from 25-day old seedlings following the procedure of Zheng et al. (1995). The PCR protocols recommended by respective authors was adopted for marker-assisted selection of *Xa21*, *Pi54*, *Rf3* and *Rf4*, respectively as given in Supplementary Table 1. Background selection was done using 62 polymorphic SSR markers following the procedure described in Sundaram et al. (2008). Using the data from polymorphic SSR markers, a schematic map illustrating the genomic contribution of donor and recurrent parents was prepared using Graphical Genotype (GGT) Version2.0. (Van Berloo 1999).

Screening of the backcross derived lines against BB and blast

Screening for BB resistance: The parents, selected BC_3F_4 lines along with TN1 (the susceptible check) and ISM (Resistant check) were screened for BB resistance through artificial clip inoculation method (Kauffman et al. 1973) during Kharif 2013 under glass house condition at ICAR-IIRR, Hyderabad, India. Four virulent isolates viz. DX-020, DX-002, DX-066 and DX-049 of *Xanthomonas oryzae* pv. *oryzae* collected from Hyderabad- Andhra Pradesh State, Faizabad- Uttar Pradesh State, Raipur- Chattisgarh State and Maruteru- Andhra Pradesh State, respectively were cultured and maintained as explained in Laha et al. (2009) and used for screening through clip inoculation protocol. The inoculated plants were scored as per standard IRRI – SES scales, 1996 (IRRI 1996) 15 days after inoculation.

Screening for blast resistance: The parents and selected BC_3F_5 lines along with HR12 (the susceptible check) and Tetep (Resistant check) were screened for blast resistance

Table 1 Reaction of the selected advanced backcross derived lines improved for resistance against bacterial blight and blast

S.No	Rice line	Resistance genes genotyped by flanking markers*				Reaction against bacterial blight#		Reaction against blast#	
		Pi54 Pi54 MAS	*Xa21* pTA248	*Rf3* DRRM-Rf3–10	*Rf4* DRCG-RF4-14	DX-020 Score	R/S	SPI-40 Score	R/MR/S
1	RPHR-1005	–	–	++	++	9.0 ± 0.0	S	9	S
2	ISM	–	++	–	–	1.7 ± 0.3	R	–	S
3	TETEP	++	–	–	–	–	–	0	R
4	RP-213-63–238-18-32-1	++	++	++	++	2.7 ± 0.7	R	1	R
5	RP-213-63–238-18-32-2	++	++	++	++	2.3 ± 0.3	R	1	R
6	RP-213-63–238-18-32-3	++	++	++	++	1.7 ± 0.3	R	1	R
7	RP-213-63–238-18-32-4	++	++	++	++	3.0 ± 0.6	R	3	R
8	RP-213-63–238-18-32-5	++	++	++	++	2.7 ± 0.7	R	3	R
9	RP-213-63–238-18-32-6	++	++	++	++	3.3 ± 0.3	R	3	R
10	RP-213-63–238-18-32-7	++	++	++	++	2.7 ± 0.3	R	2	R
11	RP-213-63–238-18-32-8	++	++	++	++	2.0 ± 0.0	R	1	R
12	RP-213-63–238-18-32-9	++	++	++	++	3.0 ± 0.6	R	1	R
13	RP-213-63–238-18-32-10	++	++	++	++	2.7 ± 0.3	R	3	R
14	RP-213-63–238-18-32-11	++	++	++	++	3.0 ± 0.6	R	3	R
15	RP-213-63–238-18-32-12	++	++	++	++	3.0 ± 0.6	R	3	R
16	RP-213-63–238-18-32-13	++	++	++	++	2.7 ± 0.3	R	3	R
17	RP-213-63–238-18-32-14	++	++	++	++	2.0 ± 0.6	R	2	R
18	RP-213-63–238-18-32-15	++	++	++	++	2.3 ± 0.3	R	1	R

*++ Possessing homozygous resistant allele at the particular gene locus, based on screening with gene-specific marker

–Possessing homozygous susceptible allele at the particular gene locus, based on screening with gene-specific marker

R, Resistant; S, Susceptible

under uniform blast nursery using SPI-40, a local isolate of the blast pathogen, *Magnaporthae oryzae* (Madhan Mohan, 2011). These lines along with resistant and susceptible checks were scored based on IRRI- SES (IRRI 1996) after 15 days of inoculation.

Evaluation of agro-morphological characters

Thirty-day old seedlings of fifteen selected BC_3F_4 lines were transplanted in the experimental farm of ICAR-IIRR, during wet season (June-November) 2013 along with the donor and recurrent parent. Data was recorded for the agronomic traits, viz. days to 50 % flowering (DFF), mean days to maturity, mean plant height (cm), number of productive panicles per plant, panicle weight (gms), panicle length (cm), grain yield per plant (gms), TG weight (gms) and grain type as explained in Sundaram et al. (2008) and Hari et al. (2013).

Generation of experimental hybrids using improved versions of RPHR-1005 and their evaluation

Three selected backcross derived lines (viz. RP-213-63–238-18-32-3, RP-213-63–238-18-32-9 and RP-213-63–238-18-32-15), possessing better panicle exsertion as compared to RPHR-1005, were crossed with the female parent of DRRH3, APMS 6 A to evaluate grain yield standard heterosis of the derived hybrids (wet season 2014). BPT5204 (Samba Mahsuri) possessing medium-slender grain type served as a varietal check, while DRRH3 served as the hybrid check.

Results

Marker-assisted simultaneous but step wise transfer of *Xa21* and *Pi54* through MABB into RPHR-1005

Out of 92 F_1 seeds generated, a total of 79 were identified to be 'true' F_1s with the help of the *Xa21*-specific marker pTA248 and *Pi54* gene-specific marker Pi54MAS and they were used for backcrossing. A total of 182 BC_1F_1 plants were grown; among these, 29 were heterozygous for both the genes (Fig. 1). When they were examined using markers specific for *Rf3* and *Rf4*, a total of six 'positive' BC_1F_1 plants (i.e. positive for the target resistance genes and homozygous for *Rf3* and *Rf4*) were identified (Fig. 1). When these plants were screened for background selection, a single plant (# RP-213)

was identified to possess a maximum recovery of recurrent parent genome (~ 71 %; as inferred from analysis involving 62 polymorphic SSR markers) and it was then backcrossed with RPHR-1005. A total of 122 BC_2F_1 plants were raised and seven among these were identified to be positive for both *Xa21* and *Pi54*. They were then subjected for background genome analysis, which revealed that a single BC_2F_1 plant (# RP-213-63) to possess a maximum of 82 % recovery of recurrent parent genome. This plant was then backcrossed to generate BC_3F_1s. Out of 346 BC_3F_1s produced 20 double positive plants (*Xa21* + *Pi54*; Supplementary Table 2) were identified and among these, single plant viz. # RP213-63–238, possessing a maximum introgression (i.e. ~ 94 %) of RPHR-1005 genome was identified through background selection. This plant was then further examined with respect to the extent of presence of donor genome with respect to the two target resistance genes, viz., *Xa21* and *Pi54.* With respect to *Xa21*, a segment of 0.6 Mb was observed to be introgressed in the north side from the donor parent genome in this plant, while in the south side, a segment of 1.0 Mb was introgressed. Thus, in total, a segment of 1.6 Mb was introgressed from the donor parent with respect to the genomic region in the vicinity of *Xa21.* With respect to *Pi54*, a segment of 0.6 Mb and 0.1 Mb were introgressed from the donor parent genome (totaling to 0.7 Mb) and the donor genome introgression is thus limited to ~2.3 Mb in the best BC_3F_1 plant (Fig. 2). This plant (# RP-213-63–238) was then selfed and a total of 40 homozygous BC_3F_2 plants were identified (based on marker screening) and advanced through pedigree method involving phenotypic trait-based selection. Finally, 15 promising, advanced backcross derived lines (ABLs) were identified at BC_3F_4 and they were subjected for phenotypic evaluation for disease resistance, yield, fertility restoration, heterosis and other agro-morphological parameters.

Assessment for blast and BB resistance of the gene-pyramid lines

The 15 ABLs possessing *Xa21* + *Pi54* were evaluated for their resistance to blast in the Uniform Blast Nursery (UBN) beds (Fig. 3, Table 1). The susceptible check, HR12 and the recurrent parent RPHR-1005 (Score-9) were highly susceptible to blast, while the resistant check, Tetep (Score-0) and all the ABLs were found to be highly resistant. With respect to bacterial blight screening (Fig. 3, Table 1), the recurrent parent, RPHR-1005 was observed to be highly susceptible to the disease with a lesion length of 15 cm, while the resistant check, ISM was observed to be highly resistant with a lesion length ranging from 1 to 3 cm. All the 15 ABLs showed high level of resistance to BB with a lesion length of 1–3 cm indicating successful introgression of *Xa21* in these lines.

Table 2 Details of agronomic performance of the parents and improved lines of RPHR-1005 under field conditions

S.No	Designation	DFF(days)	PH(cm)	NT	PL	TG(gm)	Y/P(gm)	Panicle exsertion
1	RPHR-1005	105.3 ± 0.3	90.7 ± 2.4	17.3 ± 0.3	15.7 ± 0.4	16.7 ± 0.1	27.7 ± 0.2	PE
2	RPBio Patho-2	104.7 ± 0.3	69.8 ± 0.7	16.3 ± 0.7	19.5 ± 0.5	17.1 ± 0.1	26.2 ± 0.1	PE
3	RP-213-63–238-18-32-1	100.7 ± 0.3	95.3 ± 0.6	17.7 ± 0.7	20.7 ± 0.3	17.3 ± 0.2	27.7 ± 0.2	PE
4	RP-213-63–238-18-32-2	104.7 ± 0.3	91.3 ± 1.5	15.3 ± 0.3	21.5 ± 1.1	17.9 ± 0.6	28.2 ± 0.5	FE
5	RP-213-63–238-18-32-3	93.7 ± 0.3	93.5 ± 0.6	20.7 ± 0.7	21.0 ± 0.7	18.4 ± 0.1	29.1 ± 0.6	FE
6	RP-213-63–238-18-32-4	107.3 ± 0.9	93.1 ± 0.6	19.7 ± 0.3	18.6 ± 0.4	17.5 ± 0.2	28.1 ± 0.1	FE
7	RP-213-63–238-18-32-5	106.7 ± 0.3	90.7 ± 0.3	18.0 ± 0.6	16.6 ± 0.2	17.4 ± 0.3	28.0 ± 0.6	FE
8	RP-213-63–238-18-32-6	104.3 ± 0.3	91.8 ± 1.1	17.3 ± 0.9	16.6 ± 0.8	17.1 ± 0.0	27.5 ± 0.3	FE
9	RP-213-63–238-18-32-7	101.0 ± 0.6	87.7 ± 1.4	19.0 ± 0.6	20.5 ± 0.3	17.1 ± 0.1	26.4 ± 0.4	FE
10	RP-213-63–238-18-32-8	103.0 ± 1.0	91.4 ± 0.6	17.3 ± 0.9	18.3 ± 0.7	16.8 ± 0.2	25.3 ± 0.2	FE
11	RP-213-63–238-18-32-9	93.0 ± 0.6	101.9 ± 0.5	22.3 ± 0.3	22.8 ± 0.1	18.1 ± 0.1	29.2 ± 0.4	FE
12	RP-213-63–238-18-32-10	106.3 ± 0.7	88.3 ± 0.5	16.3 ± 0.3	17.1 ± 0.2	17.6 ± 0.1	25.6 ± 0.3	E
13	RP-213-63–238-18-32-11	99.7 ± 0.7	89.0 ± 0.6	19.7 ± 0.7	20.7 ± 0.3	16.7 ± 0.4	27.1 ± 0.6	FE
14	RP-213-63–238-18-32-12	106.7 ± 0.7	91.2 ± 0.8	17.7 ± 0.7	21.7 ± 0.2	16.7 ± 0.3	24.7 ± 0.3	FE
15	RP-213-63–238-18-32-13	102.3 ± 0.9	93.9 ± 0.9	18.3 ± 0.9	16.1 ± 0.1	17.0 ± 0.1	27.7 ± 0.3	FE
16	RP-213-63–238-18-32-14	102.7 ± 0.7	86.8 ± 0.8	18.7 ± 0.7	17.1 ± 0.2	16.9 ± 0.2	26.6 ± 0.7	FE
17	RP-213-63–238-18-32-15	104.7 ± 0.7	102.3 ± 0.6	20.3 ± 0.3	22.4 ± 0.3	17.5 ± 0.1	28.4 ± 0.1	FE

DFF: Days to 50 % flowering, PH: Mean plant height (cm), NT: Number of tillers/plant, PL: Panicle length (cm), TG (gm): 1000 grain weight (gm), Y/P: Yield per plant (gm), Panicle exsertion: FE- Full exsertion, E-Exsertion, PE-Partial exsertion. ±: Standard error and values given are mean of three replications

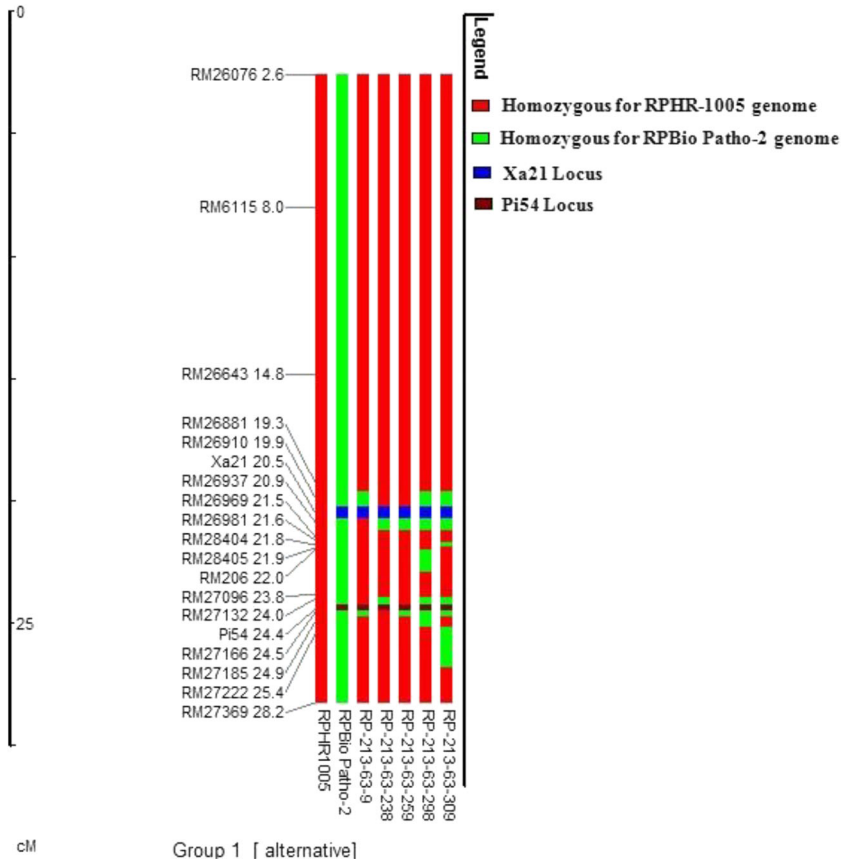

Fig. 2 Analysis of genome introgression associated with the BB resistance gene, *Xa21* & blast resistance gene, *Pi54* on chromosome 11 in improved lines of RPHR-1005 indicating a donor segment introgression, limited to ~2.3 Mb. Respect to *Xa21*, a segment of 0.6 Mb was introgressed in the north side from the donor parent genome in the best BC_3F_1 (i.e, plant # RP-213-63–238), while in the south side, a segment of 1.0 Mb was introgressed. Thus, in total, a segment of 1.6 Mb was introgressed from the donor parent with respect to the genomic region in the vicinity of *Xa21*. With respect to *Pi54*, in plant # RP-213-63–238, a segment of 0.6 Mb and 0.1 Mb were introgressed from the donor parent genome (totaling to 0.7 Mb). Thus with respect to both *Xa21* and *Pi54*, a genomic region limited to ~2.3 Mb has been only introgressed from the donor parent in the best backcross plant (i.e, plant # RP-213-63–238). The position of the polymorphic SSR markers in Mb on Chr. 11 is given in parenthesis adjacent to each marker, while each marker has also been positioned with respect to each other in terms of cM scale

Agronomic characterization of the improved lines

The ABL RP-213-63–238-18-32-1 displayed grain yield equivalent to (27.7 ± 0.2 g per plant) that of the recurrent parent (i.e. RPHR; 27.7 ± 0.2 g per plant), while other ABLs (RP-213-63–238-18-32-9 & RP-213-63–238-18-32-3) possessed grain yield per plant significantly higher than (29.2 ± 0.4 & 29.1 ± 0.6 g per plant respectively) the recurrent parent. Some of the ABLs (viz. RP-213-63–238-18-32-9 (101.9 ± 0.5 cm) & RP-213-63–238-18-32-15 (102.3 ± 0.6 cm) were observed to be taller than RPHR-1005 (90.7 ± 2.4 cm). No significant variation was observed with respect to the number of productive tillers/plant, no. of panicles and panicle length among the ABLs as compared to RPHR-1005. Two ABLs, RP-213-63–238-18-32-3 and RP-213-63–238-18-32-9 were found to flower 12–13 days earlier to that of RPHR-1005 and also possessed better panicle exsertion (Supplementary Fig. 2, Table 2).

Evaluation of heterosis of the newly derived experimental hybrids

Three elite backcross derived improved lines of RPHR-1005 possessing better panicle exsertion as compared to RPHR-1005, viz., RP-213-63–238-18-32-3, RP-213-63–238-18-32-9 and RP-213-63–238-18-32-15 with maximum parental genome recovery and also with good agro-morphological characters were test crossed with APMS 6 A, the female parent of DRRH3 in order to check the performance of newly derived hybrids vis-à-vis DRRH3 (which is derived from the cross APMS 6 A x RPHR-1005) along with the standard check (BPT5204). All the F_1s derived from respective crosses were observed to be completely fertile, indicating the three improved lines of RPHR-1005 are complete restorers. The newly derived hybrids were evaluated for their disease resistance, other parameters i.e. days to 50 % flowering (DFF), yield per plant and extent of panicle exsertion. The three

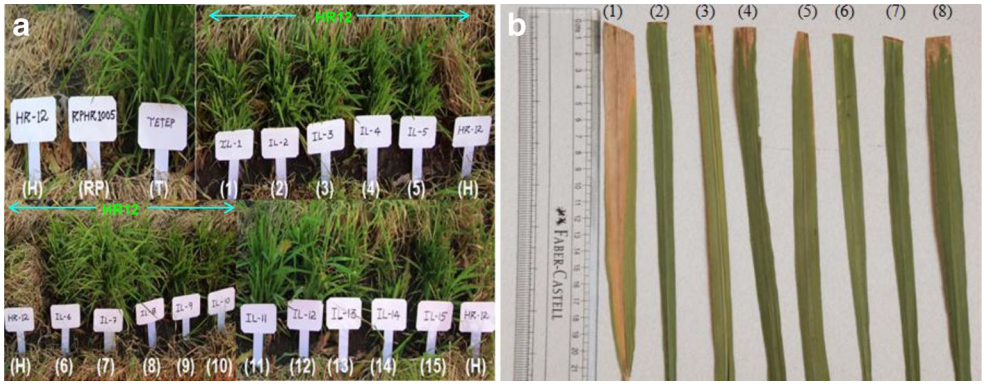

Fig. 3 Phenotypic screening of selected ABLs against rice blast and bacterial blight diseases. (**a**) Screening of the selected BC_3F_5 lines against blast disease following UBN method during Rabi 2014, (RP) RPHR-1005 – Recurrent parent (susceptible); (T) Tetep- Donor check (Resistant); (H) HR-12-Susceptible check and all the back cross derived lines IL01 to IL-15 (RP-213-63–238-18-32-1 to RP-213-63–238-18-32-15) were highly resistant to the dieases. (**b**) Scrrening parents (1) RPHR-1005 and (2) RPBio Patho-2 and selected BC_3F_4 line, viz., (3) RP-213-63-238-18-32-3(Y/E), (4) RP-213-63-238-18-32-15(HT/Y/E), (5) RP-213-63-238-18-32-9(HT/Y), (6) RP-213-63-238-18-32-1, (7) RP-213-63-238-18-32-3 and (8) RP-213-63-238-18-32-4 for resistant against bacterial blight during Kharif 2013. All the backcross derived lines were observed to be highly resistant to the diseases

newly derived hybrids (Table 3), viz. DRRH3-21(RP-213-63–238-18-32-3 X APMS 6 A), DRRH3-42 (RP-213-63–238-18-32-9 X APMS 6 A) and DRRH3-56 (RP-213-63–238-18-32-15 X APMS 6 A) and improved lines of RPHR-1005 (Table 1) displayed high level of resistance to BB with average lesion lengths of 1.0–3.0 cm and were also highly resistant to blast (1–2 score) as compared to DRRH3, which showed lesion length of 6.0 cm when screened for BB resistance and a score of 5, when screened for blast resistance. Apart from their disease resistance (Table 3) and other agronomic parameters (Table 4), two of the newly derived hybrids (i.e. DRRH3-42 and DRRH3-56) also showed higher level of heterosis (when compared to DRRH3) and all the hybrids were similar to DRRH3.

Discussion

Despite their superior grain quality and yield, DRRH3 and its parent RPHR-1005 are highly susceptible to two major rice diseases, i.e. BB and blast, which significantly reduce the yield of rice varieties and hybrids (Singh et al. 2013, Lalitha et al. 2013). Hence, the present study was carried out with an objective to improve RPHR-1005 and its derived hybrid (i.e. DRRH3) for resistance against BB and blast and improve the parental line for traits like plant height and panicle exsertion, while retaining the premium grain quality and high yield through MABB coupled with stringent phenotypic selection. We selected dominant resistance genes for introgression in the present study as introgression of dominant genes ensures that the derived hybrids will also possess resistance against the two deadly diseases of rice. Further, introgression of dominant resistance genes into the male parent (i.e. restorer line, RPHR-1005) accelerates the process of development of disease resistant hybrids as introgression of the genes into the female parent (i.e. the maintainer line or the cytoplasmic male sterile line) is cumbersome and involves several rounds of backcrosses to introgress the target genes first into the maintainer line and then later to the CMS line (Hari et al. 2013).

MABB has been demonstrated to be an efficient technique for precise transfer of one or few target genes into the genetic background of an elite variety or parental line. Earlier, Sundaram et al. (2008; 2009) and Hari et al. (2011; 2013)

Table 3 Details of reaction of the parent, improved lines of RPHR-1005 and their derived hybrids against BB and blast

S.No	Cross/genotype	Reaction against bacterial blight (DX-020)		Blast score Reaction against blast (SPI-40)	
		Score	S/MR/R	Score	S/MR/R
1	RPHR-1005	9.0 ± 0.0	S	9	S
2	Samba Mahsuri	9.0 ± 0.0	S	9	S
3	DRRH3	6.0 ± 0.6	S	5	MR
4	RP-213-63–238-18-32-3 X APMS6A (DRRH3-21)	1.7 ± 0.7	R	2	R
5	RP-213-63–238-18-32-9 X APMS6A(DRRH3-42)	1.0 ± 0.0	R	1	R
6	RP-213-63–238-18-32-15 X APMS6A (DRRH3-56)	2.3 ± 0.7	R	2	R

R Resistant; S Susceptible; MR Moderate resistance

Table 4 Details of agro-morphological traits and heterosis of the parents, improved lines of RPHR-1005 and their derived experimental hybrids (values given are mean of three replications during Kharif 2013)

S.No	Cross/genotype	PH(cm)	DFF(days)	Y/P(gm)	Grain yield heterosis over Standard check for MS BPT 5204 (%)	Grain yield heterosis over hybrid check DRRH3 (%)	Panicle exsertion
1	RPHR-1005	90.7 ± 2.4	105.3 ± 0.3	26.9 ± 0.1	-	-	E
2	Samba Mahsuri	90.3 ± 0.1	114.0 ± 0.3	15.4 ± 0.4	-	-	E
3	DRRH3 (RPHR-1005 X APMS6A)	132.3 ± 0.3	107.7 ± 0.3	21.8 ± 0.1	-	-	E
4	RP-213-63–238-18-32-3 X APMS6A (DRRH3-21)	132.7 ± 0.3	107.0 ± 0.0	21.8 ± 0.3	41.5	0	FE
5	RP-213-63–238-18-32-9 X APMS6A (DRRH3-42)	133.3 ± 0.3	108.0 ± 0.0	22.2 ± 0.2	48.0	4.5	FE
6	RP-213-63–238-18-32-15 X APMS6A (DRRH3-56)	132.0 ± 0.6	107.7 ± 0.3	22.8 ± 0.1	44.1	1.83	FE

PH Mean plant height (cm), DFF Days to 50 % flowering, Y/P Yield per plant (gm), Panicle exsertion FE- Full exsertion, E-Exsertion

developed BB resistant versions of the varieties, Samba Mahsuri and Triguna, the restorer line, KMR-3R and the maintainer line IR58025B, respectively through MABB and the same approach with modifications was adopted in the present study. Similar to our study, Basavaraj et al. (2010) improved PRR78, the restorer line of the Basmati quality rice hybrid, Pusa RH10, for resistance against BB and later Singh et al. (2012) improved it for blast resistance. However, in the present study, in addition to use of markers for selecting *Xa21* and *Pi54*, we also adopted a positive selection strategy which involved MAS for fertility restoration trait (controlled by the major genes, *Rf3* and *Rf4*) and for quick recovery of the complete RPHR-1005 genome through background selection involving just three backcrosses. When the improved backcross-derived lines of RPHR-1005 were test-crossed with popular WA-CMS line i.e. APMS 6 A, the F_1s were observed to be completely fertile, thus indicating potential use of the improved lines as restorers for developing elite, three-line hybrids.

When the stable, advanced backcrossed derived lines of RPHR-1005, possessing disease resistance and stable fertility restoration were analyzed for background genome recovery using 62 polymorphic SSR markers. As only six foreground positive plants (i.e. positive for *Xa21* + *Pi54* + *Rf4* + *Rf3*) were subjected for screening of background selection, the background genome recovery % was significantly lower than the theoretical value of 75 % at BC_1F_1 generation. However at BC_2F_1, the recurrent parent genome recovery was observed to be higher (82 %) and at BC_3F_1, the % of recovery was equivalent to the theoretical value of 93.25 % (i.e. 94 %). The principal reason why lower % of recovery of recurrent parent genome was noticed in BC_1F_1 could be due to analysis of a very low number of plants for background selection ($n = 6$). In conjunction with marker-assisted selection, we also subjected the backcross derived plants to a stringent, phenotype-based selection in the later generation so that we could recover/select some of the best phenotypic features (like good plant type, fine-grain type, increased number of grain per panicle etc. (Table 2). The analysis further indicated that donor genome segment is limited to ~2.3 Mb on either side of the target resistance genes ensuring that the advanced backcross derived plants did not have any adverse linkage drag from the donor parent. Similar results have been reported by Balachiranjeevi et al. (2015), while attempting improvement of the elite maintainer line, DRR17B for bacterial blight and blast resistance.

Through careful phenotype-based selection, we also identified a few backcross derived lines possessing nearly complete and complete panicle exsertion (Table 2) as compared to RPHR-1005. We were also able to select a few backcross lines possessing higher plant height as compared to RPHR-1005 (ideal for a good restorer line, Table 2). Some of the experimental hybrids developed by crossing such improved lines of RPHR-1005 with APMS 6 A, the female parent of DRRH3, gave higher grain yield and heterosis (Table 4) as compared to DRRH3.

In the present study, only a single dominant gene each conferring resistance against BB (i.e. *Xa21*) and blast (i.e. *Pi54*) have been selected for targeted improvement of RPHR-1005. Even though there are a few previous reports about breakdown of resistance conferred by a single BB resistance gene (Mew et al. 1992, Khush et al. 1989) in rice, till date there is no report about large-scale breakdown of resistance conferred by either *Xa21* or *Pi54* from India or abroad. Further, as per a recent report (DRR annual report, 2011–12), NILs of Samba Mahsuri and Swarna possessing only *Pi54* displayed resistance across multiple locations in India (DRR Progress report, Vol. 2, 2008–2013). When we screened the improved lines of RPHR-1005 against the predominant isolates of BB (DX-020) and blast (SPI-40), all the ABLs (Table-1) were observed to be highly resistant against both the diseases. Further, the experimental hybrids developed by crossing improved lines of RPHR-1005 with APMS 6 A also

displayed excellent resistance against BB and blast (Table 3), thus indicating that the improved versions of RPHR-1005 have good potential for development of elite MS grain type, BB and blast resistant hybrids.

Previously, Hari et al. (2013) improved the hybrid rice parental (maintainer) line IR58028B against BB and blast by introgressing (*Xa21* + *Pi54*) through MABB. Later, Basavaraj et al. (2010) and Singh et al. (2012) improved the elite restorer line PRR78 for BB and blast resistance. The present study, which has culminated in development of improved versions of RPHR-1005, like the earlier studies, stands testament to the potential of marker-assisted breeding in targeted improvement of a few selected traits of elite parental lines. The most significant achievement of this study is the near-complete recovery of the genetic background of RPHR-1005, including its stable fertility restoration feature, medium-slender grain type along with improvement with respect to selected traits like plant height and panicle exsertion (Table 2). Further, another noteworthy feature is that among the improved lines of RPHR-1005, no apparent yield penalty associated with the presence of BB (*Xa21*) & blast (*Pi54*) resistance genes was noticed. Some of the experimental hybrids derived from the cross between improved lines of RPHR-1005 and APMS 6 A were found to be superior to DRRH3 with respect to heterosis (Table 4) and all the hybrids were resistant to BB and blast (Table 3). This indicates that cultivation of the BB & blast resistant, improved parental lines and hybrids would be of great advantage in BB and blast endemic areas.

Among the improved lines of RPHR-1005, one line, RP-213-63–238-18-32-9, has been identified as best restorer line as it possesses all the good phenotypic traits of RPHR-1005 along with better plant height and near-complete panicle exsertion (Table 2). It is being used as a potential parent for developing superior hybrids by crossing it with multiple WA-CMS lines. The improved lines of RPHR-1005 (possessing BB and blast resistance along with improved panicle exsertion) and their derived hybrids are being further evaluated for their agronomic performance through station trials at IIRR, Hyderabad, India. The best hybrids will be identified and nominated shortly for multi-location trails under All India Coordinated Rice Improvement Project (AICRIP) for their evaluation and possible release for the benefit of rice farmers.

In conclusion, through the present study, we have developed improved versions of the elite restorer line, RPHR-1005 and possessing resistance against BB, blast, better panicle exsertion along with complete fertility restoration, MS grain type and demonstrated the heterotic potential of the experimental hybrids (i.e. improved versions of DRRH3) derived from crosses between improved lines of RPHR-1005 and APMS 6 A.

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Compliance with Ethical Standards

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Conflict of Interest These authors do not have any conflict of interest.

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